REMARKS

Claims 1-7 are pending in this application. Claims 1-7 have been rejected. No new matter has been added. Applicants are respectfully requesting reconsideration in view of the following remarks.

I. Rejection of the Claims Under 35 U.S.C. §103

Claims 1-7 remain rejected under 35 U.S.C. 103(a) as being unpatentable over Adrian et al. taken with Maymo-Gatell et al. in light of Bunge et al. Based upon the teachings of the cited references, the Examiner concludes:

> Therefore, it would have been obvious to one having ordinary skill in the art at the time the claimed invention was made to modify the process of Adrian *et al.* by replacing strain *Dehalococcoides* CBDB1 with strain *Dehalococcoides ethenogenes* 195 in view of their close relatedness and the teachings of Maymo-Gatell *et al.* regarding the dehalogenating properties of strain 195 for the expected benefit of providing an effective process of bioremediation for very toxic and health-damaging environmental pollutants, such as dioxins (page 3, ¶6 of the Office Action).

Applicants respectfully disagree with this conclusion and traverse this rejection. Based upon the 16S rRNA gene sequences, Adrian et al. classify CBDB1 and *Dehalococcoides ethenogenes* as distinct organisms. See Figure 2, which depicts the phylogenetic relationship of CBDB1 and *D. ethenogenes*. In this respect, Bunge et al. indicate that "*Dehalococcoides* sp. strain CBDB1 (ref 3.) is the only known bacterium able to dechlorinate chlorinated benzenes, and *D. ethenogenes* strain 195 completely dechlorinates tetrachloroethene to ethene." See page 358, ¶2. While both strain CBDB1 and strain 195 use reductive dehalogenation for energy

conservation and growth, Adrian et al. particularly point out that these two organisms have distinct metabolic requirements; the cultivation of *D. ethenogenes* requires the addition of undefined supplements, whereas the cultivation of strain CBDB1 is achieved on entirely synthetic medium. See abstract and second full paragraph, column 1, page 583. Thus, based upon the combined teachings of Adrian et al. and Bunge et al., the skilled artisan would conclude that there are clear metabolic differences between CBDB1 and *D. ethenogenes* that can not be predicted from 16S rRNA sequences. Indeed, Ward ((2002) *Proc. Natl. Acad. Sci. USA* 99:10234-10236; enclosed herewith) cautions:

> "A species is not defined by the sequence of one functional gene even if, like *amo*, it is the quintessential gene that defines the organism's metabolism. However, the organism's interaction with the environment —its regulation by environmental variables such as temperature, oxygen, and substrate concentrations— is defined at the level of functional genes and the enzymes they encode, not 16S rRNA. These are the genes that determine the role of the species, that comprise the essence of an ecological species concept. The number of functional genes for which sizeable databases are available is very small, but from these data it is obvious that the diversity of functional genes far exceeds that of the ribosome. If the diversity of functional genes reflects potential diversity in actual ecological function, then this diversity has implications for ecosystem function, resilience, and stability. In assessing the diversity of prokaryotic communities, some recognition of this additional layer of complexity must be included." See the paragraph spanning columns 2 and 3 of page 10236.

In fact, it has been demonstrated that population analysis of *Dehalococcoides* spp. using the 16S rRNA gene fails to differentiate strains because strains with the same 16S rRNA gene

sequence can have different dehalogenating abilities. See abstract of Cupples (2008) *J Microbiol Methods*. 72(1):1-11; enclosed herewith.

Applicants respectfully submit that while Adrian et al. may teach dehalogenation of aromatic chlorinated compounds with *Dehalococcoides* CBDB1, Maymo-Gatell et al. may teach that strain 195 can dehalogenate tetrachloroethene, and Bunge et al. may teach a similarity between the 16S rRNA sequence of CBDB1 and strain 195, the prior art would suggest, as evidenced by Ward and Cupples, that relatedness of 16S rRNA sequences is not predictive of the functional genes an organism may posses. Indeed, there is no evidence of record to suggest that strain 195 has the same metabolic properties of CBDB1. In fact, the cultivation requirements of these strains, as disclosed by Adrian et al., would indicate that strain 195 and CBDB1 have distinct substrate utilization profiles.

Thus, without a showing of a correspondence between ribosomal phylogeny and functional dehalogenase genes, it cannot be concluded that one can merely substitute the *Dehalococcoides* CBDB1 strain of Adrian et al. with strain 195 of Maymo-Gatell et al. with a reasonable expectation of successfully remediating a material contaminated with a halogenated aromatic compound. Accordingly, it is respectfully requested that that this rejection under 35 U.S.C. 103(a) be reconsidered and withdrawn.

II. Conclusion

Applicants believe that the foregoing comprises a full and complete response to the Office Action of record. Accordingly,

favorable reconsideration and subsequent allowance of the pending claims is earnestly solicited.

Respectfully submitted,

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